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(71) Applicant: BIODATA OY
Konalantie 6-8 A
SF-00370 Helsinki(FI)

(72) Inventor: Savolainen, Jouko Eino Tapani
Kuurinniityntie 26
SF-02700 Kauniainen(FI)

(74) Representative: Böhm, Brigitte, Dipl.-Chem., Dr. et al
Kopernikusstrasse 9, Postfach 86 08 20
W-8000 München 86(DE)

(54) Procedure for hydrolyzing keratin.

(57) The present invention relates to a procedure for hydrolyzing keratin, in which procedure the solid keratin ingredient is (a) pretreated with an aqueous solution containing sulphite ions to form denatured keratin, and (b) hydrolyzed with the aid of a proteolytic enzyme to form keratin hydrolysate. The pretreatment is carried out at about 60 to 100 °C and it lasts about 10 minutes to 4 hours. The hydrolysate thus obtained can be treated further for use as fodder addition or to form oligopeptides for use in cosmetics.

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The present invention relates to a procedure for hydrolyzing keratin, in which procedure solid keratin material is pretreated in an aqueous solution containing sulphite ions to form denatured keratin, and hydrolyzed with the aid of a proteolytic enzyme to form keratin hydrolysate. In the present context, substances containing keratin are feathers, hair, wool, bristles, horsehair, horns, cloven hooves, and hooves. Most of these, such as feathers and hair, are almost worthless as such in the industrial sense, and when waste in great masses, problematic although they decompose in the nature.

With the procedure of the invention, keratin can be hydrolyzed into a hydrolysate which the animals are able to make use of, wherewith fodder protein can be enriched with the hydrolysate.

Oligopeptides of a given length can be produced from hydrolysates for ingredients used in cosmetic products, and specifically, for treating and caring the skin and hair.

It is known that the hydrolyzability and melting of keratin-containing byproducts, e.g. feathers and hair, are enhanced when treating them physically, that is, with heat, for example, at 146 C° and at 345 kPa for 30 to 70 minutes. This so-called denaturation leads to a change in the structure of the keratin so that the sulphur bridges providing the keratin with a chemically enduring structure become open, whereby the enzymes, including the digestive enzymes of the animals, can be hydrolyzed into useful smaller ingredients, i.e. peptides, of the denatured keratin.

A drawback of the procedure is the partial destruction of certain amino acids and production of artificial amino acids, for instance lantionine, thus decreasing the quality of the produced product.

On the other hand, a chemical treatment is also known in the art for opening the sulphur bridges and for chopping the protein into smaller parts, that is, into peptides. The treatment of keratin at ≦ 2.0 to 4.0 pH, at the boiling temperature for 2 to 20 hours opens the sulphur bridges of the keratin and chops the keratin into polypeptides and oligopeptides, and even into free amino acids.

Similarly, a treatment within a highly alkaline range at the boiling temperature, and even higher, for over two hours yields the same result as mentioned above.

A drawback of the chemical treatment is the partial, or even complete destruction of certain amino acids and a highly variable size of the peptides included in the hydrolysate thus produced. The chemical hydrolysis, particularly on the alkaline side, yields artificial amino acids, lanthionine and lysinoalanine, which may be toxic.

By hydrolyzing the keratin enzymatically and by a requisite pretreatment, both taking place under mild conditions, a number of advantages are gained in comparison with the above-described methods.

The enzymatic hydrolysis requires denaturation of keratin, that is, such treatment of the native keratin that the sulphur bridges open, either all or only a certain part of them, so that the proteolytic or keratolytic enzyme is able to hydrolyze the peptide bonds of the keratin and to chop the keratin into peptides.

The pretreatment, i.e. denaturation, can be carried out physically, that is, by thermal treatment, or chemically in acid or alkaline environment, as described above, or in another chemical manner in conjunction with the hydrolysis, though under milder conditions.

In the DE-patent No. 2705669 in an enzymatic keratin hydrolysis, in which hair and wool were treated, their amount being about 7% of the dry matter, in an aqueous mixture, the denaturation has taken place at 1.5 to 2.0 pH, at ≧ 80 °C, at least for 4 hours. Thereafter the keratin-containing matter, that is, hair and wool, was water-washed.

In another DE-patent No. 3305305, an oxidative sulphitolysis is used in the denaturation, wherein the cystine of the keratin forms, together with sulphite, a keratin cysteine sulphonate and a keratin cysteine. The keratin cysteine thus produced is oxidized in the presence of a sulphite with a thionate, e.g. tetrathionate, into keratin cysteine sulphonate. The total reaction is as follows:

kerat-S-S-kerat plus 2 SO3- + $S_4O_6^-$
2 kerat-S-SO_3^- + 2 $S_2O_3^-$

The same reaction is also presented by Bailey & Cole in the publication J- Biol. Chem. 234, 17133-1739, 1959. As disclosed in the patent, the pretreatment was carried e.g. as follows: 100 g of chopped wool were treated in 3.5 liter water, the concentration being < 3%, with about 27% $NaHSO_3$ and about 24% $Na_2S_4O_6$, at 9 pH and at about 40 °C. The treatment time was 15 hours. After the treatment the wool was washed in abundant water.

The actual hydrolysis is carried out after the denaturation and wash with the aid of proteasis enzymes under mild conditions.

The hydrolysis was carried out, as disclosed in the DE-patent No. 2705669, as follows. The pretreated keratin-containing matter, i.e. hair or wool, was mixed into an about 7% mixture in 95 liter water. The temperature was maintained at 50 to 55 °C and the pH at 9.5. The mixture was stirred. A certain amount of urea and ammonia sulphate were added into the mixture. For controlling the pH, ammonia was used. The time of hydrolysis was 10 to 20 hours. For the enzyme, Bacillus proteases were employed. Upon the com-

pleted hydrolysis, the mixture was heated to 95 °C in order to destroy the enzymatic activity. The hydrolysate was filtered and the solution dried. The average molecular weight of the hydrolysate was 1000 to 3000.

The hair pretreated according to the hydrolysis disclosed in the DE-patent No. 3305305 were mixed in 3.0 liter of enzyme solution into 3.4% mixture. The pH was balanced to 9 with ammonia, and the temperature was maintained at 40 °C. For the enzyme, a commercial proteasis was used. The mixture was stirred evenly all the time. The hydrolyzing time was 3 hours. At the end of the hydrolysis, the hydrolysate was filtered, whereby the remaining components were separated. The filtrate was lyophiled (freeze-dried). Most of the peptides of the hydrolysate were in the range of 1100 to 7500 of molecular weight.

Keratin-containing byproducts, such as feathers, hair, bristles, horse hair, horns, cloven-hooves and hooves have long been regarded as almost worthless or even difficult waste. Nowadays, the amino acid composition of the keratin has been found useful, particularly regarding sulphur-containing amino acids, for enriching and diversifying the proteins of animal fodders. On the other hand, the amino acid content of keratin is equivalent to the amino acid composition of the human skin and hair, because they are, at least in part, composed of keratin. The keratin hydrolysate is therefore an appropriate and advantageous portion in cosmetic products used for caring said parts. A problem related thereto is how to hydrolyse keratin into water soluble of appropriate length or partly water soluble poly- or oligopeptides, in which the original amino acid composition will remain unchanged and which are digested in the digestive track of animals, and which, on the other hand, are appropriate for use in the cosmetic industry.

When studying the topic and in our attempts to produce a desired hydrolysate, we have come across with certain problems not solved in the above-mentioned patents, nor in any other documents known to the applicant. Surprisingly, we found that certain crucial problems, such as the small quantity of keratin-containing matter in the aqueous mixture in the pretreatment and hydrolysis stages, high salt content in the product after the hydrolysis and after the end treatment, the sulphur dioxide (SO_2) released in the final heating on the acid side, and the poly- and oligopeptides of certain dimensions obtained directly from the hydrolysis as sufficient concentrations, can be solved relatively economically without detrimental wastes and emissions.

The invention is therefore mainly characterized in what is stated in the characteristic features' part of Claim 1. Therefore, it has been understood that accelerating the sulphitolysis is possible without detrimental side effects when the temperature is raised to 60 °C. Thus, it has been possible to shorten the pretreatment from over four hours to even less than one hour.

As taught by the invention, the first part of the enzymatic keratin hydrolysis, i.e. the denaturation of the keratin, that is, opening of the sulphur bridges, is carried out chemically. It is most successful when the keratin-containing matter, such as feathers and hair, is chopped to 3 to 6 mm in an appropriate chopping machine. The chopped material is mixed in water as much as possible in a reactor. The dry-matter content of the chopped material in the mixture is advantageously about 5 to 15%. The sulphite is added into the mixture e.g. in the form of Na_2SO_3 for obtaining a sulphitolysis. The sulphite content varies from 5 to 25% of the dry matter and is mainly dependent on the desired degree of hydrolysis, the treatment temperature and time used. A potential oxidizing compound, such as copper chloride, preferably thionates, are added for an oxidative sulphitolysis, for modifying the hydrolysis degree caused by the enzyme treatment, in about zero to 20 per cent of the dry matter. According to an embodiment, the pH of the mixture is set to be in the range 6 to 9, preferably from 6.5 to 8.0. For setting the pH, NaOH, or preferably KOH and/or H_2SO_4 in preparing fodder, is used. The temperature is set from 60 to 100 °C and the treatmewnt time is from 10 minutes to 4 hours, preferably 10 to 60 minutes. The percentages are in w/w.

The second stage comprises the actual enzymatic treatment, i.e. the hydrolysis. This stage can be accomplished in the same reactor as the pretreatment because the feathers or hair need not be washed. The hydrolysis may be started directly after the pretreatment if the dry matter content is maintained appropriate. Stirring the mixture is continued. The pH is controlled preferably to be in the range 6 to 8.5. The temperature is set from 55 to 80 °C, being dependent on the enzyme used. For the enzyme, one proteolytic enzyme or a mixture of several proteolytic enzymes are used, the pH optimum whereof being neutral or slightly alkaline, and which are as resistant thermally as possible. Appropriate enzymes are the commercial neutral and alkaline proteases from different manufacturers derived from bacteria (e.g. Bacillus), from mildew (e.g. Aspergillus), and from plants and animals, or other suitable proteolytic enzymes. The selection of an enzyme or an enzyme mixture is in addition to the above requirements influenced by the length of the peptide chains of the end product. The amount of the enzyme is dispensed mainly by the amount of activity required. The amount of activity is determined mainly according to the amount of substrate

and the desired hydrolysis time and it is the greater the shorter hydrolysis time is wanted and the greater the concentration of the substrate.

After the pretreatment stage the end product of the directly continued hydrolysis, i.e. hydrolysate, remains too small in the dry matter content to serve a number of purposes, so that it has to be concentrated. This can be best influenced by adding pretreated and filtered feathers straight into the hydrolysis under way by multiple-batch-batch principle. The addition times may vary from one to three. Addition of one batch at a time is possible because after the pretreatment, the mixture of feathers and hair is easy to stir. The treatment of additional feather batches is preferably carried out in a filtrate from which the preceding feather batch has been filtered. Hereby, reactants, for instance Na_2SO_3 and $Na_2S_4O_6$, are recovered when only the missing portion of the requisite reactant amount is added for the next treatment since not all reactants are worn out in the treatment. This is preferable even for the reason that the amount of salt of the end product remains in such instances small, only 3 to 8 per cent of the dry matter content in comparison with the 10 to 15 per cent in the single batch hydrolysate, and the amount of SO_2 produced in the acid during the heating treatment of the end product remains relatively lower. The following batches are added later, as in the course of the hydrolysis the fluidity and stirrability of the mixture have again improved. In this manner, 15 to 25 per cent dry matter content of the end product is achieved.

The hydrolyzing time is determined according to the desired degree of hydrolysis when the amount of enzymatic activity and the rest of the factors are known. The most suitable hydrolysis time has proved to be 4 to 8 hours. In a multiple batch hydrolysis the use of enzyme is intensified because only in the first batch the entire "calculated" amount of the enzyme is added, and in the rest only half of the preceding one.

After the hydrolysis, the pH of the hydrolysate is set from the hydrolysis rate to 2 to 5 pH in the H_2SO_4 depending on how much of the sulphite is removed from the mixture and how much of the keratin cysteine sulphonate (Kerat-S-So_3-) is wished to be turned into the cysteine of the keratin (Kerat-S^-). After setting the pH, the mixture is heated to 70 to 100 °C for 15 to 45 minutes, whereby SO_2 is released. Most preferably the SO_2 release is carried out so that it is conducted into a pretreatment reactor to become Na_2SO_3, this being utilized in turn in the next pretreatments.

Hydrolysate meant for animal fodder is heated, preferably at 2.5 to 4.5 pH and mixed as such with fodder. The pepsine-HCl solubility of the hydrolysate thus obtained, determined as dissolved/total proteine, is 80 to 85 per cent. The salt content (Na_2SO_4) of the hydrolysate is 3 to 8 per cent of the dry matter in the multiple-batch hydrolysate and the dry matter content, 15 to 25 per cent, whereas the salt content is 10 to 15 per cent of the dry matter in a single-batch hydrolysate and 5 to 10 per cent of the dry matter.

When preparing keratin hydrolysate with oligopeptides of certain dimensions, the following procedure is so adopted that the pH of the hydrolysate, recently hydrolyzed or end-treated, is raised to 7 to 9 and the hydrolysate is heated to 80 to 90 °C for a moment, and then cooled. At that time, the proteines, precipitated at low pH, become redissolved. Thereafter the solid part is filtered off. The filtrate is microfiltered, whereby the filtrate thus obtained is sterile. Determined by the dimensions of the desired peptides the separting numbers of the ultrafilter diaphragms are selected. As an example, a sterile filtrate is ultrafiltered through a filter with a separating number of 10,000. In that instance, the permeate contains smaller than 10,000 dalton peptides. The permeate thus obtained is again ultrafiltered through a diaphragm. A keratin hydrolysate is obtained in which the dimensions of the peptides are 1,000 to 10,000 and which is at the same time concentrated to a desired concentration. In conjunction with the concentration, peptides below 1,000 dalton and other compounds and salts are washed off. In the end treatment of the hydrolysate, while heating in acid, the cysteine sulphonates of the oligopeptides become respective cysteines with the extraction of SO_2. In conjunction with ultrafiltration, while washing the concentrate, the salt content reduces. Then sulphur bridges are again produced between the cysteines of the peptides in the alkaline and oxidizing environment. The concentration of the end product is stabilized along with the ultrafiltering. The end product may also be dry, whereby the concentrate is dried in the form of freeze drying, jet drying or vacuum drying.

The following examples illustrate the above-described invention.

Example 1

215 kg of chopped feathers, dry weight about 60 kg, were mixed into a uniform mixture of 735 liter in a 2,000 liter reactor. 4.8 kg of Na_2SO_3 were added into the mixture and the pH was set to 7.5. Stirring all the time the mixture was heated to 95 °C and it was maintained thereat for 40 minutes.

After the above treatment the mixture was cooled to 60 °C and the pH was set to 8.0, and it was maintained at that with KOH. For the enzyme used in the hyrdrolysis, a proteolytical bacteria-derived enzyme (Maxatase, Gist-Brocades, Netherlands)

was used. The mixture was stirred continuously so that the entire mass was moving and became well mixed.

The hydrolysis lasted 6 hours. Thereafter the pH was set to 4.0 with H_2SO_4. Simultaneously, about 25 liter per minute of filtered air were conducted through the reactor. The SO_2 thus produced was in this manner conducted to a second reactor in which it was dissolved in water in the form of Na_2SO_3. After the thermal treatment the hydrolysate was cooled to 60 °C and packed into plastic containers.

The raw proteine of the hydrolysate of the dry matter was 80.5% and ash, 14.5%, and the pepsine HCl solubility (soluble/total proteine), was 82%.

Example 2

1.0 kg of chopped feathers of 28% dry matter content was mixed in water so that 28.0 g of Na_2O_3 and 23.0 g of $Na_2S_4O_6$ were added in the mixture. The pH was set to 8.0. The mixture was heated to 80 °C and it was maintained at that for 30 minutes while stirring.

After the treatment the mixture was cooled to 65 °C and pH was set to 7.0 and it was maintained at that with NaOH. For the enzyme, papaine was used (Profix, Biocon, Ireland), its amount being so calculated that 4 hours were enough for the hydrolyzing time. The mixture was stirred strongly throughout the hydrolysis. Upon the hydrolysis, the pH of the mixture was maintained at 7.0, and it was heated to 90 °C for a moment for destroying the enzymatic activity. Thereafter the mixture was cooled to 35 °C. The solid ingredients were filtered off from the mixture and the clear filtrate was ultrafiltered with diaphragms of 10,000 separating number. Only a very small portion became concentrated during the filtering, and therefore, most of it was filtered through as a permeate. In the next stage the permeate thus obtained was concentrated and washed with ultrafiltering, the separating number of the diaphragms thereof being 1,000. The concentrate was washed until all penetrating peptides were removed and the salt content was one tenth of the original.

Finally, the hydrolysate was so concentrated that the end product was a semifluid and rather light solution. Part of the above solution was freezedried in small bottles.

Example 3

1.5 kg of chopped pig bristles were mixed in 5.0 liter water. 40.0 g of $NaHSO_3$ and 10.0 g of $Na_2S_4O_6$ were added in the mixture. The pH was set to 6.5 and the mixture was heated to 90 °C and maintained at that for 30 minutes. After the denaturation the mixture was cooled to 60 °C and the pH was set to 7.0 and maintained thereat with KOH. For the enzyme was used a mixture containing two thirds of bacteria-originated proteolytic enxyme (Alcalase, Novo, Denmark) and one third of papaine (Profix, Biocon, Ireland). They were added separately. Such amount of the enzyme mixture was added that the hydrolysis lasted 4 hours. The hair mixture was mixed throughout the hydrolysis.

Upon the1 completed hydrolysis, the pH of the mixture was still maintained at 7.0. The mixture was heated for a moment to 90 'C in order to inactivate the enzyme and cooled tp 35 °C. The treatment of the hydrolysate into end products was carried out as in Example 3.

Example 4

1.0 kg chopped feathers (dry matter 27%) were mixed in 3 l of water and so much of water was added so that the total volume was 5.0 l. The mixture was stirred efficiently. 30.0 g of Na_2SO_3 was added in the mixture, the pH was set to 7.5. The mixture was heated to 95 °C and maintained thereat for 30 minutes. After the denaturation the mixture was cooled to 60 °C and the solid part filtered off from the solution. The solid part was stored for the hydrolysis.

The filtered solution, i.e. filtrate, was returned to the reactor and a second 1.0 kg batch of the same feathers and water were added so much that the end volume of the mixture was 5.0 liter. The mixture was stirred and 15.0 g of Na_2SO_3 were added because the filtrate contained already free SO_3^-. The mixture was hot-treated and filtered in the same way as the first batch. The filtrate of the second batch was returned again into the reactor and a third batch, one kilo of the same feathers as before and so much water were added that the end volume of the mixture was 5.0 liter. Na_2SO_3 was added and it was treated in the same way as the second batch.

The hydrolysis of the denatured feathers was carried out so that 1.0 liter of the filtrate of the third treatment batch was added in the reactor, and the solid part of the feathers of the same batch, separated by filtering, was added by stirring efficiently. In order to ensure the mixing, 0.5 liter of the same filtrate was added, the pH was set to 7.5 and it was maintained at that value with NaOH. For the enzyme a commercial proteolytic enzyme (Alcalase, Novo, Denmark) was used and so much of it was added that the hydrolysis took place in 4 hours. After one hour of the hydrolysis, the filtered solid part of feathers of the second pretreatment batch was added. The hydrolysis was continued under the same conditions. Half of the enzyme amount of

the second batch was added. After the last addition, the hydrolysis was continued for 4 hours. After the hydrolysis, the hydrolysate was treated as in Example 1, with the exception that SO_2 was not removed by means of air current. In the finished hydrolysate the dry matter content was 19% and the ash content of the dry matter, 8.5%.

Claims

1. A procedure for hydrolyzing keratin, in which the solid keratin ingredient is
   (a) pretreated in an aqueous solution containing sulphite ions into denatured keratin, and
   (b) hydrolyzed with the aid of a proteolytic enzyme into keratin hydrolysate,
   characterized in that the pretreatment is carried out at 6 to 9 pH, at about 60 to 100 °C, lasting from about 10 minutes to 4 hours, and that the hydrolysation of stage (b) is carried out by feeding the denatured keratin ingredient in several steps into the hydrolysis mixture.

2. Procedure according to claim 1, characterized in that the pretreatment is carried out at 6.5 to 8 pH.

3. Procedure according to claim 1 or 2, characterized in that the pretreatment (a) is carried out at 75 to 100 °C.

4. Procedure according to claim 1, 2 or 3, characterized in that the pretreatment lasts from about 10 to 60 minutes.

5. Procedure according to claim 1, 2, 3 or 4, characterized in that the pretreatment (a) is carried out in such an aqueous solution containing sulphite ions which contains sodium sulphite Na_2SO_3.

6. Procedure according to any one of the preceding claims, characterized in that the amount of sodium sulphite is in the range 5 to 25 per cent of the dry keratin ingredient weight.

7. Procedure according to claim 1, 2 or 3, characterized in that the pretreatment (a) is carried out in such aqueous solution containing sulphite ions which contains sodium sulphite Na_2SO_3 and an alkali metal thionate, preferably sodium tetrathionate $Na_2S_4O_6$.

8. Procedure according to any one of the preceding claims, characterized in that the pretreatment (a) is carried out so that the dry matter content of the keratin ingredient in the aqueous solution containing sulphite ions is about 5 to 15 per cent by weight.

9. Procedure according to any one of the preceding claims, characterized in that the aqueous solution of the same stage (a) containing sulphite ions is used for the pretreatment of several keratin ingredient batches.

10. Procedure according to any one of the preceding claims, characterized in that in the finished pretreatment mixture of stage (a) the hydrolysation of stage (b) is carried out as such.

11. Procedure according to any one of the preceding claims, characterized in that for the proteolytic enzyme an enzyme or enzymatic mixture is used in stage (b) in which the pH optimum is neutral or somewhat alkaline and which is thermally resistive.

12. Procedure according to claim 10, characterized in that the enzyme is a neutral and/or alkaline protease derived from bacteria, such as Bacillus, from mildew, such as Aspergillus, from a plant or an animal.

13. Procedure according to any one of the preceding claims, characterized in that the temperature of the hydrolysis stage (b) is set to about 55 to 80 °C.

14. Procedure according to any one of the preceding claims, characterized in that the pH value of the hydrolysis stage (b) is set to about 6 to 8.5.

15. Procedure according to any one of the preceding claims, characterized in that it comprises stage (c) in which the pH of the mixture hydrolyzed in stage (b) is set to value of about 2 to 5 with sulphuric acid H_2SO_4 and is heated to about 70 to 100 °C for about 15 to 45 minutes.

16. Procedure according to claim 15, characterized in that the mixture obtained in stage (c) is heated in stage (d_1) when the pH is in the range of about 2.5 to 4.5, and it is mixed in animal fodder for use as protein addition.

17. Procedure according to claim 1 to 14, characterized in that the pH of the mixture obtained in stage (b) is in stage (d_2) raised to value of about 7 to 9, and it is heated for a moment to about 80 to 90 °C, whereafter the potential solid ingredient is filtered off for producing

oligopeptides of a given dimension e.g. for cosmetic mixtures.

18. Procedure according to claim 17, characterized in that the SO_2 released during stage (d_1) is conducted to stage (a) together with an alkaline for producing sulphite.

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(71) Applicant: **BIODATA OY**
Konalantie 6-8 A
SF-00370 Helsinki(FI)

(72) Inventor: **Savolainen, Jouko Eino Tapani**
Kuurinniityntie 26
SF-02700 Kauniainen(FI)

(74) Representative: **Böhm, Brigitte, Dipl.-Chem., Dr. et al**
Kopernikusstrasse 9, Postfach 86 08 20
W-8000 München 86 (DE)

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EUROPEAN SEARCH REPORT

Application Number

EP 92 10 2441

DOCUMENTS CONSIDERED TO BE RELEVANT

Category	Citation of document with indication, where appropriate, of relevant passages	Relevant to claim	CLASSIFICATION OF THE APPLICATION (Int. Cl.5)
Y	DE-A-3 539 231 (LICENCIA) * claims 1,6 * ---	1-18	C08H1/06 C12S13/00 A61K7/06 A23K1/10
Y	DE-A-1 962 207 (MONSANTO) * claims 2,6 * ---	1-18	
X D	GB-A-2 115 427 (L'OREAL) & DE-A-3 305 305 * example 1 * -----	1-18	

TECHNICAL FIELDS SEARCHED (Int. Cl.5)

C08H
A61K
A23K
C12R

The present search report has been drawn up for all claims

Place of search	Date of completion of the search	Examiner
THE HAGUE	17 MAY 1993	LENTZ J.C.

CATEGORY OF CITED DOCUMENTS

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